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Development of an Organic Controlled Environment Vitrification System to Expand Cryo-Protection of Beam Sensitive Materials

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Cryogenic Transmission Electron Microscopy (Cryo-TEM) has been of huge importance in the structural biology field [4]. The development of a controlled environment vitrification system (CEVS) [1] allowed beam sensitive, solvated structures such as proteins to be imaged. Commercial CEVS plunging systems allow for systemic, and repeatable sample preparation using water based samples. The key feature of the CEVS is that the sample blotting can occur in a high water humidity environment which prevents evaporation. This has been shown to be essential for preventing rearrangement of the structures inside the solution, However, many material syntheses do not take place in water. While there have been examples of cryo-TEM being performed in non-aqueous solutions, or mixture of organic and aqueous solutions [4], as far as we can tell there is no commercially available or in-house CEVS which has been designed to be compatible with organic solvents. This is especially important for samples containing volatile organic solvents which evaporate quickly after blotting. In our hands, we have found that the preparation of samples using toluene, dioxane, tetrahydrofuran, acetone, ethanol etc. result in the rearrangement of structures due to solvent evaporation using a conventional CEVS [3,5]. Consequently, we believe that the development of an organic CEVS (OCEVS) will allow the Cryo-TEM study of a broad range of materials in organic solvents or solvent mixtures.

Our OCEVS (Figure 1) has been designed using Delrin for its structural rigidity and chemical resistance. Key components include: a temperature controlled solvent reservoir to adjust vapor pressure inside the OCEVS. The solvent reservoir includes a glass sight and intake sight to allow ease of filling inside the glove box. It is also detachable to allow for easier cleaning, making it possible to use solvents with lower vapor pressure with no fear of further contamination. Temperature control of the environment, monitored by a thermocouple located near the TEM grid, separate from the solvent reservoir to allow for cooling and heating studies. The plunge mechanism uses a double action pneumatic piston which provides automated, and repeatable plunging action. The blotter uses an analog feedback servo to allow for constant monitoring of its position. The system is controlled by an ESP32 microcontroller programmed in house. Using the ESP32 as an access point, we created a simple and intuitive user interface to allow for input of thermodynamic data to aid in the solvent vaporization process, as well as blotter timing parameters. Importantly, the environment will be oxygen free as the OCEVS will be placed inside a glove box with an inert atmosphere for safety reasons and to prevent any unwanted oxygen or air interactions from occurring. For this reason, the device (Figure 1a) will be inside the glove box along with 3 buttons for plunging, deplunging, and lighting controls. And the touch screen interface (Figure 1b) will be outside the glove box. For ease of use with multiple samples to be prepped, the interface allows for input of information for any number of sequential samples to be prepped, provided the same solvent mixture is used so no clean up is required. This allows the researcher to input the parameters all at once, and never needing to remove the bulky gloves except at the end of the preparation of all their samples.

We will use the DDBSP - Dortmund Data Bank Software Package to calculate the temperature needed to create the desired atmosphere composition.

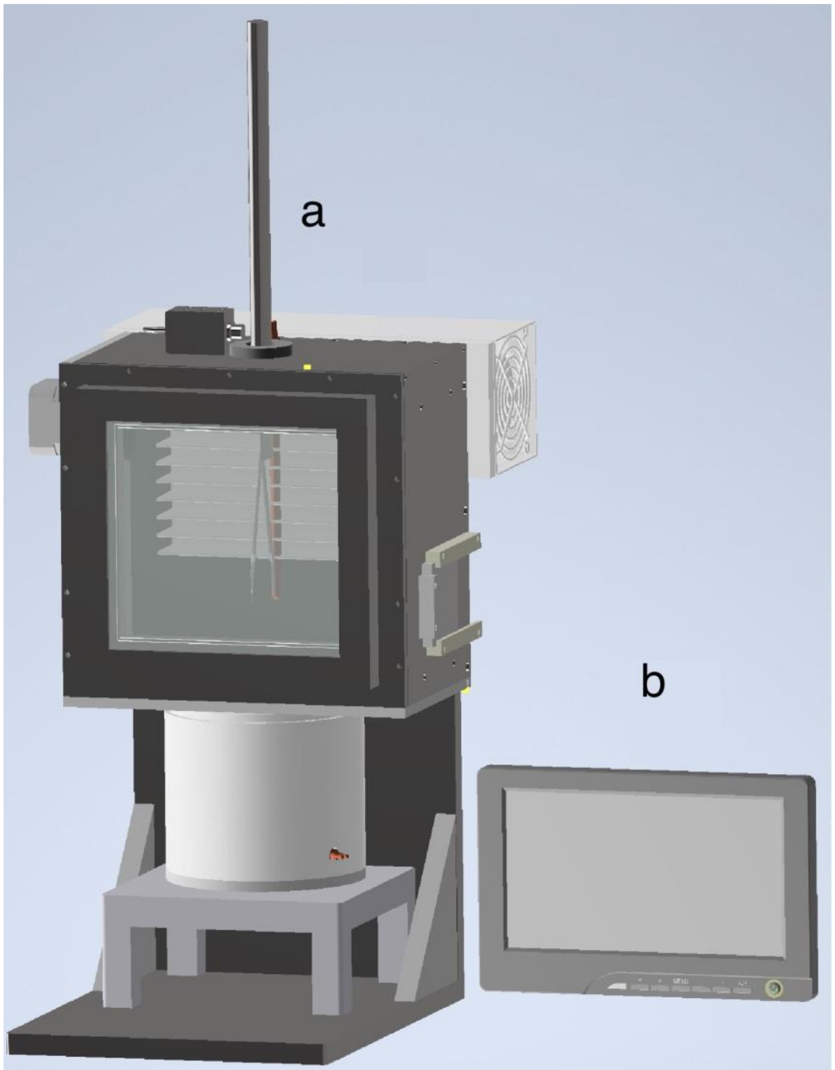

Figure 1. An image from our computer-aided design of the organi controlled vitrification system OCEVS showing the a) main body of the OCEVS including the nitrogen dewar and stand and b) the touch screen interface for control of operation.

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